

Claims:

1. An assay method for detecting fungal infection of soil or vegetables by pathogenic fungal species, in particular *M. acerina*, *F. carotae* and *Pythium* species, said method comprising:

5 obtaining a sample of soil or vegetable; treating said sample to lyse fungal cells therein; using an oligonucleotide primer pair, effecting a polymerase chain reaction on DNA released by lysis of the fungal cells; and detecting DNA fragments generated by said polymerase chain reaction;

10 wherein said primer pair comprises an 18- to 24-mer having the ability to hybridize to one of the oligonucleotide sequences of formulae Ia (SEQ ID NO:1), Ib (SEQ ID NO:2), IIa (SEQ ID NO:3), IIb (SEQ ID NO:4), IIIa (SEQ ID NO:5), IIIb (SEQ ID NO:6), IVa (SEQ ID NO:7), IVb (SEQ ID NO:8), Va (SEQ ID NO:9), Vb (SEQ ID NO:10), VIa (SEQ ID NO:11), VIb (SEQ ID NO:12), VIIa (SEQ ID NO:13), VIIb (SEQ ID NO:14), VIIIa (SEQ ID NO:15), VIIIb (SEQ ID NO:16), IXa (SEQ ID NO:17), IXb (SEQ ID NO:18), Xa (SEQ ID NO:19), Xb (SEQ ID NO:20), XIa (SEQ ID NO:21), XIb (SEQ ID NO:22), XIIa (SEQ ID NO:23), XIIb (SEQ ID NO:24), XIIIa (SEQ ID NO:25), XIIIb (SEQ ID NO:26), XIVa (SEQ ID NO:27) and XIVb (SEQ ID NO:28):

5'	- TCA CTT GTG GGG TAA AGA AGA - 3'	(Ia)
5'	- AGA CCA CAA TAA AGC GGC - 3'	(Ib)
30	5' - AGT CCC GCA CAC ACA CAT - 3'	(IIa)
5'	- ACT TCT CTC TTT GGG GAG TGG - 3'	(IIb)
5'	- TTC GTT CAG CCT CTG CAT - 3'	(IIIa)
5'	- TCG TTT CGG CTA TGA ATA CAG - 3'	(IIIb)
5'	- ACA AAT ATA CCA ACC ACA GCG - 3'	(IVa)
35	5' - TTT GTA CTT GTG CAA TTG GC - 3'	(IVb)
5'	- AAC GAA TAT ACC AAC CGC TG - 3'	(Va)
5'	- TCA TCT ATT TGT GCA CTT CTT TTT - 3'	(Vb)

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5'	- TCT TCT TTA CCC CAC AAG TGA	- 3'	(VIa)
5'	- GCC GCT TTA TTG TGG TCT	- 3'	(VIb)
5'	- ATG TGT GTG TGC GGG ACT	- 3'	(VIIa)
5'	- CCA CTC CCC AAA GAG AGA AGT	- 3'	(VIIb)
5	5' - ATG CAG AGG CTG AAC GAA	- 3'	(VIIIa)
	5' - CTG TAT TCA TAG CCG AAA CGA	- 3'	(VIIIb)
	5' - CGC TGT GGT TGG TAT ATT TGT	- 3'	(IXa)
	5' - GCC AAT TGC ACA AGT ACA AA	- 3'	(IXb)
	5' - CAG CGG TTG GTA TAT TCG TT	- 3'	(Xa)
10	5' - AAA AAG AAG TGC ACA AAT AGA TGA - 3'		(Xb)
	5' - GTT TGA ATG GAG TCC GAC CG - 3'		(XIa)
	5' - CGG CGT ACT TGC TTC GGA GC - 3'		(XIb)
	5' - TGG GAT TAA CGG GCA GAG AC - 3'		(XIIa)
	5' - TTT CGC ATT CGG AGG CTT GG - 3'		(XIIb)
15	5' - CGG TCG GAC TCC ATT CAA AC - 3'		(XIIIa)
	5' - GCT CCG AAG CAA GTA CGC CG - 3'		(XIIIb)
	5' - GTC TCT GCC CGT TAA TCC CA - 3'		(XIVa)
	5' - CCA AGC CTC CGA ATG CGA AA - 3'		(XIVb)

20 2. A method as claimed in claim 1 for detecting fungal infection of soil by pathogenic *Pythium* species, said method comprising:
 obtaining a sample of soil; treating said sample to lyse fungal cells therein; using an oligonucleotide primer pair, effecting a polymerase chain reaction on DNA released by lysis of the fungal cells; and detecting DNA fragments generated by said polymerase chain reaction; wherein said primer pair comprises an 18- to 24-mer, having the ability to hybridize to one of the
 25 oligonucleotide sequences of formulae Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIa, VIIIb, IXa, IXb, Xa and Xb:

30	5' - TCA CTT GTG GGG TAA AGA AGA - 3'	(Ia)
35	5' - AGA CCA CAA TAA AGC GGC - 3'	(Ib)
	5' - AGT CCC GCA CAC ACA CAT - 3'	(IIa)
	5' - ACT TCT CTC TTT GGG GAG TGG - 3'	(IIb)

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5'	- TTC GTT CAG CCT CTG CAT - 3'	(IIIa)
5'	- TCG TTT CGG CTA TGA ATA CAG - 3'	(IIIb)
5'	- ACA AAT ATA CCA ACC ACA GCG - 3'	(IVa)
5'	- TTT GTA CTT GTG CAA TTG GC - 3'	(IVb)
5	5' - AAC GAA TAT ACC AAC CGC TG - 3'	(Va)
	5' - TCA TCT ATT TGT GCA CTT CTT TTT - 3'	(Vb)
	5' - TCT TCT TTA CCC CAC AAG TGA - 3'	(VIa)
	5' - GCC GCT TTA TTG TGG TCT - 3'	(VIb)
	5' - ATG TGT GTG TGC GGG ACT - 3'	(VIIa)
10	5' - CCA CTC CCC AAA GAG AGA AGT - 3'	(VIIb)
	5' - ATG CAG AGG CTG AAC GAA - 3'	(VIIIa)
	5' - CTG TAT TCA TAG CCG AAA CGA - 3'	(VIIIb)
	5' - CGC TGT GGT TGG TAT ATT TGT - 3'	(IXa)
	5' - GCC AAT TGC ACA AGT ACA AA - 3'	(IXb)
15	5' - CAG CGG TTG GTA TAT TCG TT - 3'	(Xa)
	5' - AAA AAG AAG TGC ACA AAT AGA TGA - 3'	(Xb)

3. A method as claimed in claim 1 for detecting fungal infection of soil or vegetables by pathogenic fungal species, said method comprising:

20 obtaining a sample of soil or vegetable; treating said sample to lyse fungal cells therein; using an oligonucleotide primer pair, effecting a polymerase chain reaction on DNA released by lysis of the fungal cells; and detecting DNA fragments generated by said polymerase chain reaction;

25 wherein said primer pair comprises an 18- to 24-mer having the ability to hybridize to one of the oligonucleotide sequences of formulae XIa, XIb, XIIa and XIIb, XIIIa, XIIIb, XIVa and XIVb:

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5'	- GTT TGA ATG GAG TCC GAC CG - 3'	(XIa)
5'	- CGG CGT ACT TGC TTC GGA GC - 3'	(XIb)
5'	- TGG GAT TAA CGG GCA GAG AC - 3'	(XIIa)
35	5' - TTT CGC ATT CGG AGG CTT GG - 3'	(XIIb)
	5' - CGG TCG GAC TCC ATT CAA AC - 3'	(XIIIa)
	5' - GCT CCG AAG CAA GTA CGC CG - 3'	(XIIIb)

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5' - GTC TCT GCC CGT TAA TCC CA - 3' (XIVa)
5' - CCA AGC CTC CGA ATG CGA AA - 3' (XIVb).

4. A method as claimed in claim 2 wherein said primer
5 pair comprises a pair of 18- to 24-mers having the
ability to hybridize to a pair of the oligonucleotide
sequences of formulae Ia and Ib or IIa and IIb or IIIa
and IIIb or IVa and IVb or Va and Vb.

10 5. A method as claimed in claim 3 wherein said primer
pair comprises a pair of 18- to 24-mers having the
ability to hybridize to a pair of the oligonucleotide
sequences of formulae XIa and XIb or XIIa and XIIb.

15 6. An assay method for detecting fungal infection of
soil or vegetables by pathogenic fungal species, in
particular *M. acerina*, *F. carotae* and *Pythium* species,
said method comprising:

20 obtaining a sample of soil or vegetable; treating said
sample to lyse fungal cells therein; using an
oligonucleotide primer pair, effecting a polymerase
chain reaction on DNA released by lysis of the fungal
cells; contacting the DNA fragments generated by said
polymerase chain reaction with a substrate having
25 immobilized thereon a primer which comprises an 18- to
24-mer having the ability to hybridize to one of the
oligonucleotide sequences of formulae Ia, Ib, IIa, IIb,
IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb,
VIIIa, VIIIb, IXa, IXb, Xa, Xb, XIa, XIb, XIIa, XIIb,
30 XIIIa, XIIIb, XIVa and XIVb:

5' - TCA CTT GTG GGG TAA AGA AGA - 3' (Ia)
5' - AGA CCA CAA TAA AGC GGC - 3' (Ib)
5' - AGT CCC GCA CAC ACA CAT - 3' (IIa)
35 5' - ACT TCT CTC TTT GGG GAG TGG - 3' (IIb)
5' - TTC GTT CAG CCT CTG CAT - 3' (IIIa)
5' - TCG TTT CGG CTA TGA ATA CAG - 3' (IIIb)

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5' - ACA AAT ATA CCA ACC ACA GCG - 3' (IVa)
 5' - TTT GTA CTT GTG CAA TTG GC - 3' (IVb)
 5' - AAC GAA TAT ACC AAC CGC TG - 3' (Va)
 5' - TCA TCT ATT TGT GCA CTT CTT TTT - 3' (Vb)
 5 5' - TCT TCT TTA CCC CAC AAG TGA - 3' (VIa)
 5' - GCC GCT TTA TTG TGG TCT - 3' (VIb)
 5' - ATG TGT GTG TGC GGG ACT - 3' (VIIa)
 5' - CCA CTC CCC AAA GAG AGA AGT - 3' (VIIb)
 5' - ATG CAG AGG CTG AAC GAA - 3' (VIIIa)
 10 5' - CTG TAT TCA TAG CCG AAA CGA - 3' (VIIIb)
 5' - CGC TGT GGT TGG TAT ATT TGT - 3' (IXa)
 5' - GCC AAT TGC ACA AGT ACA AA - 3' (IXb)
 5' - CAG CGG TTG GTA TAT TCG TT - 3' (Xa)
 5' - AAA AAG AAG TGC ACA AAT AGA TGA - 3' (Xb)
 15 5' - GTT TGA ATG GAG TCC GAC CG - 3' (XIa)
 5' - CGG CGT ACT TGC TTC GGA GC - 3' (XIb)
 5' - TGG GAT TAA CGG GCA GAG AC - 3' (XIIa)
 5' - TTT CGC ATT CGG AGG CTT GG - 3' (XIIb)
 5' - CGG TCG GAC TCC ATT CAA AC - 3' (XIIIa)
 20 5' - GCT CCG AAG CAA GTA CGC CG - 3' (XIIIb)
 5' - GTC TCT GCC CGT TAA TCC CA - 3' (XIVa)
 5' - CCA AGC CTC CGA ATG CGA AA - 3' (XIVb);
 and detecting DNA fragments binding to said primer.

25 7. An 18- to 24-mer oligonucleotide primer
 hybridizable to an oligonucleotide sequence selected
 from those of formulae Ia, Ib, IIa, IIb, IIIa, IIIb,
 IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIa, VIIIb,
 IXa, IXb, Xa, Xb, XIa, XIb, XIIa, XIIb, XIIIa, XIIIb,
 30 XIVa and XIVb.

8. A primer as claimed in claim 7 hybridizable to an
 oligonucleotide sequence selected from those of formulae
 Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa,
 35 VIb, VIIa, VIIb, VIIIa, VIIIb, IXa, IXb, Xa and Xb.

9. A primer as claimed in claim 7 hybridizable to an

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oligonucleotide sequence selected from those of formulae XIa, XIb, XIIa, XIIb, XIIIa, XIIIb, XIVa and XIVb.

10. A primer as claimed in claim 7 wherein said primer
5 comprises a sequence of formulae Ia, Ib, IIa, IIb, IIIa,
IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIa,
VIIIb, IXa, IXb, Xa, Xb, XIa, XIb, XIIa, XIIb, XIIIa,
XIIIb, XIVa or XIVb or a derivative thereof.

10 11. A substrate having immobilized thereon at least one
18- to 24-mer oligonucleotide primer hybridizable to an
oligonucleotide sequence selected from those of formulae
Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa,
VIb, VIIa, VIIb, VIIIa, VIIIb, IXa, IXb, Xa, Xb, XIa,
15 XIb, XIIA, XIIb, XIIIa, XIIIb, XIVa and XIVb.

12. A substrate as claimed in claim 11 wherein said
primer comprises a sequence of formulae Ia, Ib, IIa,
IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa, VIb, VIIa, VIIb,
20 VIIIa, VIIIb, IXa, IXb, Xa, Xb, XIa, XIb, XIIa, XIIb,
XIIIa, XIIIb, XIVa or XIVb or a derivative thereof.

13. A primer composition comprising a pair of 18- to
24-mer oligonucleotide primers at least one of which is
25 hybridizable to an oligonucleotide sequence of formula
Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, Va, Vb, VIa,
VIb, VIIa, VIIb, VIIIa, VIIIb, IXa, IXb, Xa, Xb, XIa,
XIIb, XIIa, XII, XIIIa, XIIIb, XIVa or XIVb optionally
30 together with a carrier.

30 14. A primer composition as claimed in claim 13 wherein
at least one of said pair is a primer comprising a
sequence of formulae Ia, Ib, IIa, IIb, IIIa, IIIb, IVa,
IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIa, VIIIb, IXa,
35 IXb, Xa, Xb, XIa, XIb, XIIa, XIIb, XIIIa, XIIIb, XIVa or
XIVb or a derivative thereof.

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15. A composition as claimed in claim 13 comprising a pair of 18- to 24-mer oligonucleotide primers at least one of which is hybridizable to an oligonucleotide sequence of formula Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, 5 IVb, Va, Vb, VIa, VIb, VIIa, VIIb, VIIIa, VIIIb, IXa, IXb, Xa or Xb.

10 16. A composition as claimed in claim 13 comprising a pair of 18- to 24-mer oligonucleotide primers at least one of which is hybridizable to an oligonucleotide sequence of formulae XIa, XIb, XIIa, XIIb, XIIIa, XIIIb, XIVa or XIVb.

15 17. A kit for the performance of the assay method of any one of claims 1 to 5, said kit comprising at least one primer pair as defined in any one of claims 1 to 5 together with instructions for the performance of the assay method.

20 18. A process for the extraction of nucleic acid from soil which process comprises:

- 1) contact a sample of about 0.1 to 1g, preferably about 0.5g, soil taken from a mixed sample of at least 100g, preferably at least 200g, soil with a fungal cell lysing agent;
- 2) centrifuge at least 10000xg for at least 10 minutes and collect the supernatant;
- 3) contact the supernatant with a particulate DNA-binding agent;
- 30 4) centrifuge and collect the DNA-bearing particulate;
- 5) suspend the particulate in an aqueous solution of a chaotropic agent (e.g. aqueous guanidine thiocyanate solution), centrifuge and collect the DNA-bearing particulate;
- 35 6) repeat step (5) at least once;
- 7) suspend the particulate in aqueous salt/ethanol wash solution, centrifuge and collect the DNA-

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- 8) bearing particulate;
- 9) repeat step (7) at least once;
- 10) suspend the particulate in an aqueous solution of a DNA-release agent;
- 5 11) centrifuge and collect the DNA-containing supernatant; and optionally
- 10 12) resuspend the particulate in an aqueous solution of a DNA-release agent, centrifuge and collect and combine the supernatant.

19. A kit for nucleic acid extraction from soil, which kit comprises:

- i) an aqueous fungal cell lysing agent;
- 15 ii) a DNA-binding particulate;
- iii) an aqueous solution of a chaotropic agent (e.g. guanidine thiocyanate);
- iv) an aqueous solution of salt and ethanol; and
- v) an aqueous solution of a DNA-release agent;

20 together with instructions for the use of said kit in the process of claim 13.

20. A process for the extraction of pathogen DNA from host vegetable tissue, which process comprises:

- 25 i) contact at least 20 mg of dry powdered plant tissue (preferably surface tissue such as peel) with at least 5 μ L/mg dry tissue of an aqueous fungal cell lysing agent;
- 30 ii) incubate;
- iii) mix with at least 4.5 μ L/mg dry tissue of an aqueous solution of a protein and polysaccharide precipitating agent;
- iv) centrifuge and collect DNA-containing supernatant;
- 35 v) filter;
- vi) contact DNA-containing filtrate with a DNA-

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binding substrate and centrifuge;

vii) wash the DNA-carrying substrate with an aqueous ethanolic solution, centrifuge and remove the liquid phase;

5 viii) repeat step (vii) at least once;

ix) dry the DNA-carrying substrate; and

x) contact the substrate with an aqueous solution of a DNA release agent, centrifuge and collect the DNA-containing supernatant.

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21. A kit for pathogen DNA extraction from host vegetable tissue, which kit comprises:

a) a fungal cell lysing agent;

15 b) an aqueous solution of a protein and polysaccharide precipitating solution;

c) a DNA-binding substrate;

d) an aqueous ethanolic wash solution; and

e) an aqueous solution of a DNA release agent;

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together with instructions for the use of said kit for pathogen DNA extraction from host vegetable tissue.